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FEBS Letters 581 (2007) 2811–2819

Minireview

The peroxisomal protein import machinery

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Received 2 March 2007; revised 27 March 2007; accepted 2 April 2007

Available online 9 April 2007

Edited by Horst Feldmann

Abstract **Peroxisomes are unique organelles whose physiological functions vary depending on the cellular environment or metabolic and developmental state of the organism. These changes in enzyme content are accomplished by the dynamically operating membrane and matrix protein import machineries of peroxisomes that rely on the concerted function of at least 20 peroxins. The import of folded matrix proteins is mediated by cycling receptors that shuttle between the cytosol and peroxisomal lumen. Receptor release back to the cytosol represents the ATP-dependent step of peroxisomal matrix protein import, which consists of two energy-consuming reactions: receptor ubiquitination and dislocation.**
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Keywords: Peroxisome biogenesis; Protein targeting; PEX; Peroxin; Ubiquitination; AAA ATPases

1. Introduction

Peroxisomes are single-membrane-bound organelles that fulfil a large variety of functions in virtually all eukaryotic cells. They exhibit a pronounced morphological and metabolic plasticity, which is dependent on the organism, cell type and prevailing environmental conditions.

Peroxisomes were initially described as ''microbodies'' in electron microscopic pictures of mouse kidney cells [1]. Subsequently, de Duve developed the Nobel Prize honoured differential and gradient centrifugation method for cell-fractionation [2]. These tools were instrumental for the isolation of an organelle fraction containing catalase and hydrogen peroxide producing oxidases which was named peroxisomes [3].

Peroxisomes appear to own a unique variability in enzyme content and thus metabolic function, which mark them as ''multi-purpose organelles'' that are adjusted according to the cells needs [4]. The enzymes in the peroxisomal matrix are linked to different biochemical pathways. A central function is the β-oxidation of fatty acids and the detoxification of the thereby produced hydrogen peroxide. This pathway is exclusively localized in the peroxisomal compartment of fungi and plants, whereas in mammalian cells the breakdown of different types of fatty acids is distributed between peroxisomes and mitochondria [5]. The decomposition of fatty acids mark peroxisomes as one source of signalling molecules such as reactive oxygene species (ROS) in plants [6] or lipid ligands for the peroxisome proliferator-activated receptors (PPAR) family in humans, whose dysfunction is linked to hepatocellular carcinoma [7]. The enzymes of the peroxisomal β-oxidation pathway are also involved in the synthesis of chemical compounds which function as phytohormones in plants, such as jasmonates or indol-3-acetic acid [6]. Other functions include α-oxidation of branched-chain fatty acids in mammals and plants [8], the main reactions of photorespiration in leaf peroxisomes [9], the final steps of penicillin biosynthesis in some filamentous fungi [10], or synthesis of bile acid and ether lipids such as plasmalogens, which contribute more than 80% of the phospholipid content of the white matter in the brain [11]. The generation and detoxification of hydrogen peroxide and other ROS relates peroxisomes to the molecular process of aging [12].

Specialized forms of peroxisomes were initially described as separate organelles. In this context, the glyoxysomes of plant seedlings and some fungi house enzymes of the glyoxylate cycle that enable the conversion of lipids into carbohydrates [13]. Filamentous fungi contain Woronin-bodies additionally to other microbodies. Their task is to seal septal pores in their hyphae in order to prevent fatal cytosolic bleeding [14,15]. The glycosomes of trypanosomes harbour key enzymes of glycolysis [16] which might also be true for the microbodies of the pathogenic basidiomycete *Cryptococcus neoformans* [17].

The study of peroxisomal biogenesis and protein import was hampered for a long time by their great fragility and low abundance in many tissues. This situation changed when it was discovered that peroxisome proliferation can be induced in bakers yeast by manipulation of the carbon source [18]. When *S. cerevisiae* cells were grown on oleic acid as the sole carbon source, peroxisomes become essential for growth because they represent the exclusive site for fatty acid degradation in yeast. This allowed the screening of mutants affected in the biogenesis of peroxisomes, referred to as *pex* mutants [19,20] with *PEX* being the acronym for the corresponding gene, the gene products were collectively named peroxins [21]. To date 32 peroxins are known [22,23]. They are involved in the three key stages of peroxisomal development: (i) formation of the peroxisomal membrane, (ii) peroxisome proliferation and (iii) compartmentalization of peroxisomal matrix proteins.

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Abbreviations: AAA, ATPase associated with various cellular activities; ERAD, endoplasmatic reticulum associated degradation; PTS, peroxisomal targeting signal; RING, really interesting new gene; Ub, ubiquitin

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doi:10.1016/j.febslet.2007.04.001

2. Peroxisome formation and inheritance

The origin of the peroxisomal membrane has been a matter of debate for a long time. Early studies which were based on ultrastructural investigations using electron microscopy and suggested that peroxisomes generate by budding from the endoplasmic reticulum (ER) [24]. Later, biochemical data demonstrated that peroxisomal matrix proteins are synthesized on free ribosomes in the cytosol and that these proteins are imported posttranslationally in pre-existing peroxisomes. Based on these results, an ER-independent model, termed "growth and division model", was proposed, which predicted that peroxisomes multiply autonomously like mitochondria or chloroplasts [25]. Although the sum of the published data placed peroxisomes among these autonomously multiplying organelles, the observation that the reintroduction of a gene into a peroxisome-lacking deletion strain, could induce a de novo formation of peroxisomes, remained difficult to explain. Recent studies, mainly based on real-time fluorescence microscopy combined with biochemical approaches, provided evidence for the ER being the source for the origin of peroxisomal membranes, at least during de novo formation [23,26]. This process requires the integral membrane protein Pex3p, which is localized to the ER at first, concentrates in foci that bud off in a Pex19p-dependent manner and mature to functional peroxisomes [26]. Peroxisome formation in mammalian cells also depends on the function of cotranslationally inserted Pex16p [27]. Little is known about the ER-targeting of Pex3p to the ER as well as the budding and subsequent maturation of peroxisomes. In *Yarrowia lipolytica*, this process involves formation and fusion of pre-peroxisomal vesicles which is thought to depend on the ATPase associated with various cellular activities (AAA) proteins Pex1p and Pex6p [28].

Based on data from plant and mammalian cells, a retrograde pathway of membrane portions from the peroxisome to the ER is discussed as well [29,30]. As a consequence of the recent results, peroxisomes are believed to constitute a semi-autonomous part of the secretory pathway [26] (Fig. 1).

However, new peroxisomes are believed to arise primarily by duplication of the pre-existing peroxisomes. To this end, peroxisomes contain an elaborate fission and proliferation machinery. Pex11p was among the first components of this device which were discovered. The deletion of *PEX11* leads to a strong reduction of peroxisome number together with an increase in size of the remaining peroxisomes. Additionally, the Pex11p-type peroxins Pex25p and Pex27p play a role in controlling size and number of *S. cerevisiae* peroxisomes. Their function is thought to induce constriction of the organelle [31] (Fig. 1). Pex25p is also described as recruitment factor for the GTPase Rho1p [32] which controls actin reorganisation at the peroxisomal membrane and thus may be required for the peroxisomal division and the inheritance process. The scission

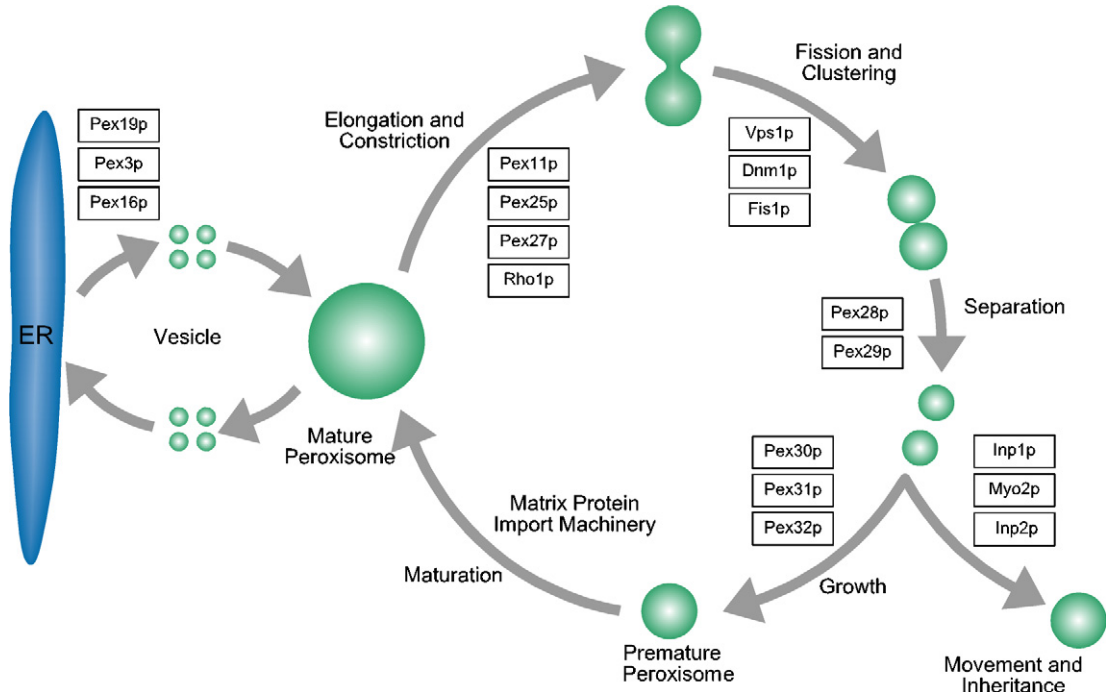

Fig. 1. Model for the division and proliferation of peroxisomes. Pex19p, Pex3p and Pex16p are required for the formation of the peroxisomal membrane as they facilitate the insertion of peroxisomal membrane proteins. This explains their initial requirement for the de novo formation of peroxisomes at the ER. The formation of mature peroxisomes may involve the fusion of precursor vesicles, as observed in *Y. lipolytica*. The peroxisomal membrane proteins Pex11p, Pex25p and Pex27p are involved in the elongation and constriction of mature peroxisome. The GTPase Rho1p might be involved in this process because it binds to Pex25p. Consecutively, the peroxisome undergoes a fission event, which requires the dynamin-related proteins Vps1p(DLP1) and Dnm1p. The former is anchored to the peroxisomal membrane via Fis1p. The divided but clustered peroxisomes are supposed to be separated by Pex28p and Pex29p, which are the orthologues of *Y. lipolytica* Pex24p. The size of peroxisomes is thought to be regulated by Pex30p, Pex31p and Pex32p, which are the orthologues of *Y. lipolytica* Pex23p. The maturation process of the peroxisome is facilitated by the import of matrix proteins. Inheritance of peroxisomes requires Inp1p, Inp2p and Myo2p. The nomenclature refers to the proteins from *S. cerevisiae*, with the exception of Pex16p, which has not yet been identified in bakers yeast but is known to exist in humans, plants and other fungi.

of peroxisomal membranes has recently been connected to the function of the dynamin-related proteins Vps1p(DLP1) and Dnm1p, as well as the Vps1p-anchoring protein Fis1p, all of which are also involved in fission processes of mitochondria [23]. The peroxisomes are still clustered after the fission event. These clusters are thought to be dissociated by the membrane proteins Pex28p and Pex29p of *S. cerevisiae*, respectively their orthologue Pex24p in *Y. lipolytica* [33]. The size of peroxisomes is further regulated by Pex30p, Pex31p and Pex32p in *S. cerevisiae* or the orthologous Pex23p from *Y. lipolytica* [22]. These peroxins act downstream of Pex28p and Pex29p, but not much is known about their molecular function.

The movement of peroxisomes into daughter cells and thus peroxisomal inheritance is regulated by Inp1p, which is supposed to link peroxisomes to a cortical anchor to retain them in mother cell and bud. Inp2p is a peroxisomal receptor for Myo2p which moves peroxisomes along polarized actin cables into the bud of the dividing cell [34].

3. Topogenesis of peroxisomal membrane proteins

Insertion of peroxisomal membrane proteins (PMPs) requires Pex3p, Pex19p and in some organisms Pex16p [35] (Fig. 2). Pex19p is supposed to function as a soluble chaperon and import receptor for PMPs. This is based on the finding that most PMPs contain conserved Pex19p-binding sites which almost always are also required for their peroxisomal localization and protein stability [36,37]. For proper peroxisomal targeting, integral peroxisomal membrane proteins in addition require an adjacent transmembrane domain [37], peripheral membrane proteins require the intact binding site to their anchoring protein [38]. Accordingly, the signal sequence for the targeting to the peroxisomal membrane (mPTS) constitutes of the Pex19p-binding site plus a membrane anchoring sequence which can be a transmembrane segment or a protein binding site.

In addition to the Pex19p-dependent targeting pathway, which directs peroxins directly to the peroxisomal membrane (class I proteins), a second insertion pathway has been proposed for Pex3p and Pex16p (class II proteins). It is believed, that theses proteins travel via a so far uncharacterized region of the ER. Pex3p binds Pex19p at the peroxisomal membrane or at the ER and thus may function as the membrane anchor of cargo-loaded Pex19p [39] (Fig. 2). Pex16p is thought to serve as a recruitment factor for Pex3p or as a component of the putative membrane translocase [27]. Although the exact mechanism of PMP insertion into the peroxisomal membrane remains to be investigated, the described functions of Pex3p and Pex19p in this process are generally accepted. However, some other observations are not that easy to reconcile with this model, as *pex19*Δ cells of *Y. lipolytica* still contain peroxisome-like structures [40] or the finding that peroxisomes can be formed independently of Pex19p from the ER in *Hansenula polymorpha* [41].

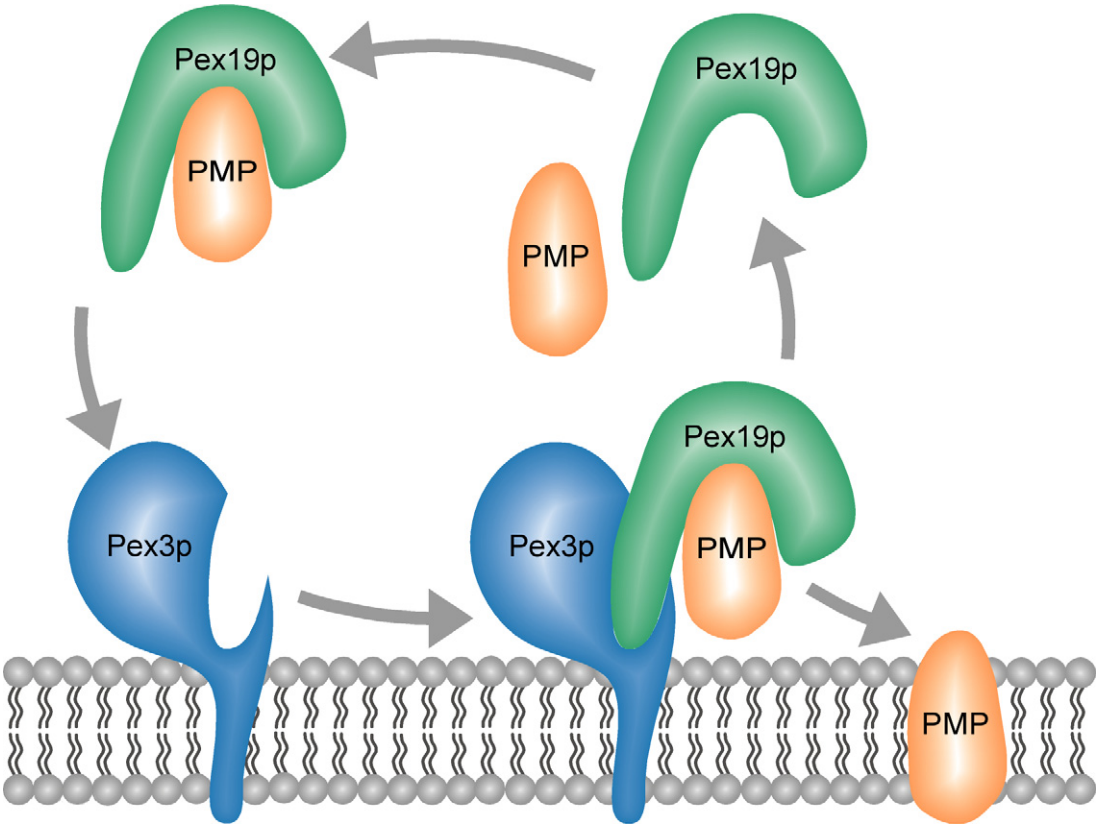

Fig. 2. Topogenesis of peroxisomal membrane proteins. Most peroxisomal membrane proteins (PMPs) contain a peroxisomal membrane targeting signal (mPTS) that is recognized by the import receptor and chaperon Pex19p which directs its cargo to the peroxisomal membrane or alternatively to the ER during de novo formation of peroxisomes. Pex3p functions as membrane anchor protein for the Pex19p-PMP complexes at the ER and peroxisomes.

4. Peroxisomal matrix protein import

One remarkable feature of peroxisomes is the fact that they can import fully folded and even oligomeric proteins. They share this ability with the nucleus and the TAT translocase of thylacoides and bacteria but this feature distinguishes them from mitochondria, chloroplasts and the ER [42]. Peroxisomes do not contain DNA. All peroxisomal matrix proteins are encoded in the nucleus, synthesized on free ribosomes and imported posttranslationally [25]. Besides formation of the protein import machinery at the peroxisomal membrane, another prerequisite for protein import is the recognition of the matrix proteins by dynamic receptors in the cytosol. The peroxisomal protein import conceptually can be divided in four steps. First, soluble receptors bind their cargo proteins in the cytosol and guide them to a docking site at the peroxisomal membrane (i), the receptor–cargo complex translocates to the luminal site of the peroxisomal membrane (ii), where the complex is disassembled in order to release the cargo (iii) and the receptor is returned to the cytosol (iv) (Fig. 3).

4.1. Targeting signal-dependent cargo recognition

The targeting of proteins destined for import into peroxisomes relies on two conserved peroxisomal targeting signals. The majority of peroxisomal matrix proteins possess a peroxisomal targeting signal type 1 (PTS1) at the very carboxy-terminus consisting of the tripeptide sequence SKL or variants thereof [43,44]. Pex5p interacts with the signal predominantly via six tetratricopeptide repeats (TPRs) within its carboxy-terminal half. Crystal structures of the receptor in absence and presence of cargo revealed major conformational changes within Pex5p upon substrate binding [45]. Interestingly, the cargo is bound to the receptor not only by the PTS1-sequence but also by a topologically separate interaction site [45]. It is an open question how this secondary interface contributes to the efficiency of the protein import.

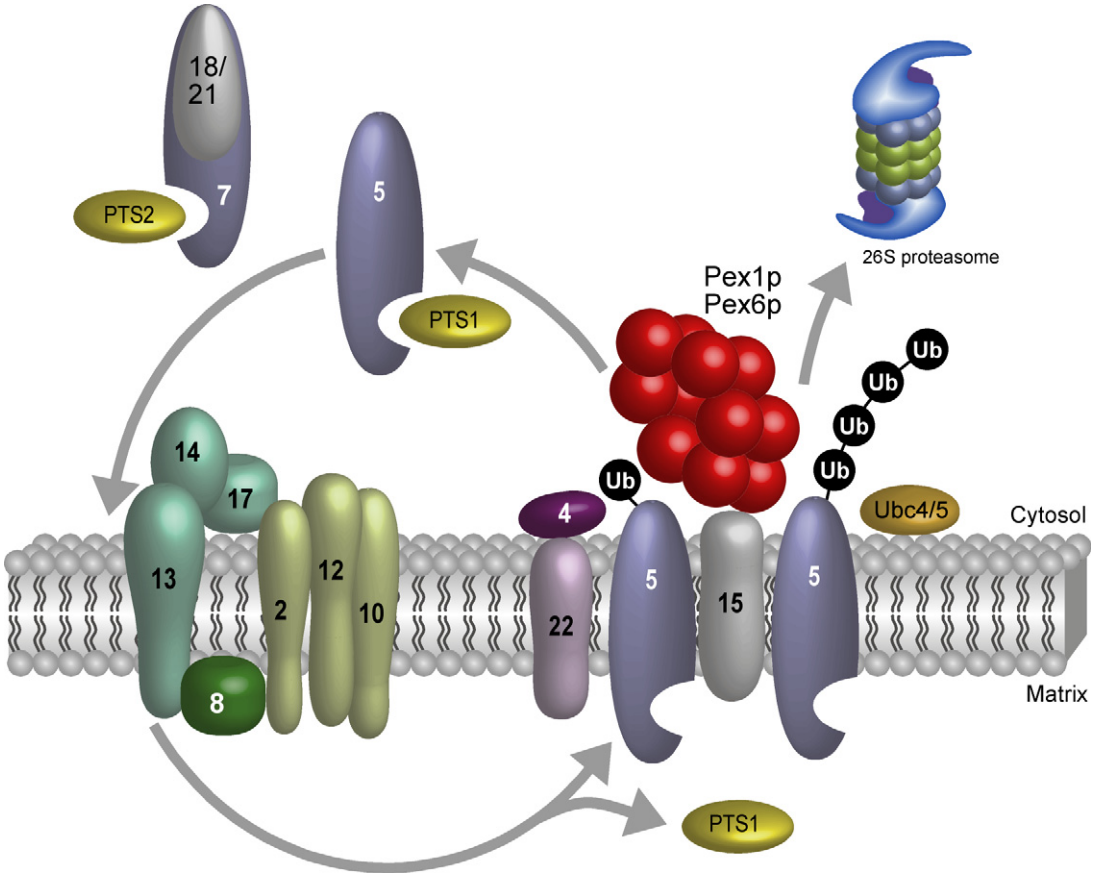

Fig. 3. Peroxisomal matrix protein import cascade. The peroxisomal protein import conceptually can be divided in four steps: (i) cargo recognition in the cytosol and direction of the receptor–cargo complexes to the peroxisomal membrane. (ii) Translocation of the receptor–cargo complex to the luminal site of the peroxisomal membrane. (iii) Disassembly of the receptor–cargo complex in the peroxisomal lumen and (iv) return of the receptor to the cytosol. Proteins harbouring the peroxisomal targeting signal type 1 are recognized by the soluble receptor Pex5p. PTS2 proteins are recognized by Pex7p, which cooperates with Pex18p and Pex21p in *S. cerevisiae*, the orthologous Pex20p in other fungi or Pex5L in plants and mammals. The receptor–cargo complexes associate with the peroxisomal membrane via the peroxisomal docking complex, consisting of Pex14p, Pex13p and Pex17p. The RING-finger domain containing peroxins Pex2p, Pex10p and Pex12p form the RING-finger complex, which is connected to the docking-complex via Pex8p. The receptor–cargo complex dissociates at the luminal site of the membrane. The integral PTS1-receptor is either monoubiquitinated by the E2-enzyme Pex4p or polyubiquitinated by Ubc4p or Ubc5p. The AAA peroxins Pex1p and Pex6p, which are anchored to the peroxisomal membrane by Pex15p in yeast or its orthologue Pex26p in mammals, dislocate the ubiquitinated Pex5p from the membrane back to the cytosol. The polyubiquitinated PTS1-receptors are degraded by the proteasome, whereas the monoubiquitinated receptors are recycled for further rounds of import.

The PTS2 is a nona-peptide with the consensus sequence (R/K)/(L/V/I)X5(H(Q))(L/A) localized near the amino-terminus of matrix proteins [46]. PTS2-harbouring proteins are recognized by the WD40 protein Pex7p [47]. The PTS2 pathway displays a higher variability than the PTS1 pathway in terms of the receptor complexes involved. Pex7p cooperates with the redundant co-receptors Pex18p and Pex21p in *S. cerevisiae* or the orthologous Pex20p proteins in other fungi species [48] which are required for the proper targeting and stabilization of the receptor–cargo complexes. In mammalian cells, a short and a long isoform of Pex5p have been identified, termed Pex5S and Pex5L. These proteins differ only in a short insertion in Pex5L, which has been demonstrated to bind Pex7p. Furthermore, it has been shown that both PTS pathways are also coupled in plants. Thus, whereas in yeast and fungi both PTS pathways converge at the membrane-bound protein import machinery, they coincide in higher eukaryotes already at the level of Pex5L [48].

Interestingly, some proteins, like acyl-CoA oxidases from different species contains no obvious PTS signal although their import still depends on Pex5p. These non-PTS proteins bind to separate binding sites of the receptor that are distinct from the TPR domain [49].

Although the PTS1- and PTS2-recognition factors differ in some aspects, they are characterized by a similar modular architecture. Accordingly, the co-receptor/Pex7p-complex of the PTS2-pathway resembles the Pex5p of the PTS1-pathway in many aspects. The PTS2-cargo recognition factor Pex7p is functionally related to the C-terminal part of Pex5p, which comprises the cargo binding region while in terms of structure and function the co-receptors are related to the N-terminal part of Pex5p. This region is responsible for the peroxisomal targeting and association with different components of the translocation machinery. The resemblance of the corresponding factors is obvious by chimera of Pex18p (without its Pex7p-binding site) fused to the TPR-domains of Pex5p which can rescue PTS1-import in a *PEX5*-deficient strain [50].

4.2. Docking of the receptor–cargo complex at the peroxisomal membrane

The three peroxins Pex13p, Pex14p and Pex17p constitute the docking complex of the peroxisomal import machinery for incoming receptor–cargo complexes [49]. Pex17p is a peripheral membrane protein of unknown function which associates to peroxisomes via Pex14p. Interestingly, in silico research results predict Pex17p to be genetically fused to Pex14p in a number of species [51]. Both Pex13p and Pex14p interact with each other and both proteins also bind Pex5p. Pex13p is an integral membrane protein containing a Src homology (SH3) domain, which provides a binding site for the proline-rich SH3-ligand motif (PXXP) in Pex14p. However, Pex13p and Pex14p contain several additional binding sites for Pex5p, which are partially embedded in the membrane, pointing to a very dynamic interaction modus [52,53]. The amount of PTS-receptors at peroxisomal membrane remnants is reduced in *pex14*Δ mutant cells in comparison to *pex13*Δ or other mutants [54] and cargo-loaded Pex5p exhibits a higher binding affinity to Pex14p than to Pex13p [55]. Thus, Pex14p is believed to make the first contact of the PTS-receptors within the complex network of protein–protein interactions upon cargo translocation across the peroxisomal membrane.

4.3. Translocation and cargo release

Several components of the peroxisomal protein import machinery bind the import receptors upon cargo translocation, giving rise to the idea of an import cascade in which the receptors are contacted by one component after the other. The mechanism of protein translocation across the peroxisomal membrane and release of cargo into the lumen is not clear and the composition of the translocon has not yet been elucidated. Components of the docking complex themselves might constitute part of the translocon [49]. One interesting fact about Pex5p is that it changes its membrane topology during the protein import cascade. First, it is soluble in the cytosol but at the peroxisomal membrane it behaves like an integral membrane protein [56]. At the end of the import cascade, Pex5p reaches the luminal side of the peroxisomal membrane [57], although it is still a matter of debate whether the whole receptor–cargo complex (“extended shuttle hypothesis”) or just a part of Pex5p (“simple shuttle hypothesis”) reaches the peroxisomal lumen upon cargo translocation [58]. Equally, Pex7p has been demonstrated to behave like a cycling receptor [59] and it is likely that also its co-receptor Pex20p enters the peroxisome [60]. According to the “transient pore hypothesis” [61], the receptors and co-receptors might contribute to a dynamic import pore which opens the membrane dynamically for a second species of cargo-loaded Pex5p and Pex7p, respectively. In this respect, it is interesting to note that recombinant Pex5p might insert spontaneously into phospholipid membranes [53], however, conclusive evidence for the existence of such a pore is still missing. Likewise, the mechanism of cargo release inside the peroxisome is not understood. In this context, the functional role of Pex8p, an intraperoxisomal peripheral membrane protein, is discussed controversially. Pex8p contains both the PTS1- as well as the PTS2-sequence that may either function in disassembling the receptor–cargo complexes or in targeting of Pex8p to the peroxisomes [62]. The best understood function of Pex8p relates to the connection of the docking complex to the peroxisomal really interesting new gene (RING)-finger complex, which is composed of the RING-motif containing peroxins Pex2p, Pex10p and Pex12p [63]. The multi-protein complex consisting of docking- and RING-complex is called the “importomer” [63]. The function of the RING-finger peroxins is not known to date, but they were often correlated with cargo translocation. This is mainly based on the findings that disruption of the RING-complex inhibits the import of Pex5p [64–66], whereas others find Pex5p [67] and Pex20p [60] to accumulate inside the peroxisome under these conditions.

After release into the matrix of mammalian, plant and *Y. lipolytica* peroxisomes, the PTS2-signal sequences are proteolytically removed from most proteins [49]. Recently, the corresponding peptidase of mammals has been identified as Tysnd1 [68]. Processing of the peroxisomal signal sequences does not seem to occur in *S. cerevisiae*.

4.4. Receptor ubiquitination and recycling

Subsequent to cargo liberation, Pex5p, Pex7p and Pex20p are exported back to the cytosol for further rounds of import [57,59,60].

Early studies discovered that the peroxisomal matrix protein import is an energy-dependent process requiring the hydrolysis of ATP [69]. Investigations in permeabilized cell systems of

human fibroblasts provided first evidence that Pex5p accumulated reversibly at the peroxisomal membrane under conditions when protein transport was blocked [65]. Detailed in vitro studies revealed that the binding and translocation of Pex5p itself is ATP-independent while the export of Pex5p back to the cytosol requires ATP [70]. The identity of the corresponding ATPase remained a matter of speculation until in vitro systems in *S. cerevisiae* [54] and human fibroblast cells [66] identified the peroxisomal AAA ATPases Pex1p and Pex6p as the motor-proteins of Pex5p export. Their function is not redundant and depends on the presence of their membrane anchor, Pex15p in yeast and its orthologue Pex26p in mammalian cells. In vitro reconstitution of the complete Pex5p cycle revealed that ATP-binding and hydrolysis in the conserved domains of both Pex1p and Pex6p were needed for the receptor dislocation [54]. The binding and consumption of ATP is believed to induce conformational changes that generate the driving force to pull the receptor out of the membrane.

The exact mechanism of substrate recognition by the AAA peroxins is not well understood. Although Pex5p and the AAA ATPases form a complex at the peroxisomal membrane [54,66,71], no direct interaction of the PTS-receptors with either Pex1p or Pex6p has yet been reported. This interaction seems to be regulated or mediated by a third factor, which could represent an unknown adaptor protein of the AAA peroxins or posttranslational modification of the substrate. It is well known that both parameter play a central role in the function of the AAA protein Cdc48p(p97,VCP) [72], which is the evolutionary closest relative of Pex1p and Pex6p [73]. As a consequence, the question has to be addressed of how the AAA peroxins can distinguish Pex5p forms destined for dislocation from cargo-loaded Pex5p species.

The X-ray structure of the N-terminal domain of Pex1p revealed striking similarities to the corresponding domains of other AAA proteins like Cdc48p(p97,VCP) or Sec18p(NSF) and demonstrates the existence of a double-psi β-barrel fold [74]. This structural feature was interpreted as a putative adaptor binding site. Recent data from Cdc48p and Ufd1p identify this fold as a ubiquitin-binding domain with two binding sites for mono- and polyubiquitin, respectively [75]. Most interestingly, the PTS-receptors Pex5p, Pex18p and Pex20p are ubiquitinated and this modification plays a functional role in their receptor cycle [60,76,77]. The PTS1-receptor Pex5p of *S. cerevisiae* has been demonstrated to be monoubiquitinated in wild-type cells of *S. cerevisiae* [78] and was shown to be polyubiquitinated in mutants of the AAA- and Pex4p/Pex22p-complexes in *S. cerevisiae* and *H. polymorpha* [76,79,80]. The PTS2-co-receptor Pex20p of *P. pastoris* is polyubiquitinated in the same set of mutants [60].

Polyubiquitination of the PTS1 receptor Pex5p requires the ubiquitin conjugating enzymes Ubc4p [76,78,79] and the partly redundant Ubc5p [76] and Ubc1p [78], exclusively takes place at the peroxisomal membrane at the end of the receptor cycle and primes the protein for proteasomal disposal. Apparently the efficiency of proteasomal disposal of the receptor varies among different species significantly. While Pex5p from *S. cerevisiae* is heavily polyubiquitinated in recycling mutants, single deletions in the same set of proteins result in a dramatic decrease of the Pex5p steady state concentration in human cells [65], plants [81], *H. polymorpha* [82,83] and *P. pastoris* [84]. Polyubiquitination of Pex5p and Pex20p is pronounced in *pex* mutants whose normal function is connected to receptor recycling and can be classified as a part of a quality control system [54,79]. Alternatively, the acronym RADAR (*receptor accumulation and degradation in absence of recycling*) has been suggested [60]. This mechanism is supposed to reconstitute binding capacities for cargo-loaded receptors at the peroxisomal membrane by removing dysfunctional receptor molecules [60,61].

Although the AAA peroxins can release polyubiquitinated Pex5p from the membrane, polyubiquitination of the PTS1-receptor itself does not seem to be a prerequisite for the export process under physiological conditions [85]. However, the PTS1-receptor Pex5p can be transiently modified by monoubiquitination which often is associated with signalling or protein trafficking rather than proteasomal degradation [86]. Monoubiquitination of Pex5p takes place at the peroxisomal membrane and depends on an intact importomer [78]. Moreover, it is independent from Ubc4p, Ubc5p or Ubc1p [78] and from all non-peroxisomal E2-enzymes but requires Pex4p (Ubc10p) [85], an E2-enzyme which is essential for peroxisomal biogenesis [87] and anchored via Pex22p to the peroxisomal membrane [88]. Pex5p is a molecular target for Pex4p (Ubc10p)-dependent monoubiquitination and either poly- or monoubiquitination of the receptor is required for the ATP-dependent release of the protein from the peroxisomal membrane to the cytosol as part of the receptor cycle [85]. Thus, monoubiquitination of Pex5p can be regarded as the export signal under physiological conditions. Polyubiquitination seems to provide an export signal for the release of dysfunctional or accumulating PTS1-receptor from the membrane as part of the quality control pathway.

The target residue for monoubiquitination remains to be identified. A clue to the identity of the target residue may be drawn from the recent observation that the conserved cysteine residue of Pex20p from *P. pastoris*, which can be found in all PTS1- and PTS2-co-receptors, is required for the recycling [89]. Ubiquitin usually forms an isopeptide bond with the ε-amino group of an internal lysine residue. Another possibility is the conjugation of ubiquitin to the α-amino group of the N-terminus of a protein. However, also the formation of a thioester bond with a cysteine residue of the target protein is possible [86]. The requirement for the conserved cysteine for recycling which also depends on ubiquitination opens the possibility that the monoubiquitination of Pex5p occurs at the conserved cysteine residue or that this residue is needed for the ubiquitination of another lysine or the N-terminus of Pex5p. While it seems clear that the purpose of monoubiquitination is to prime Pex5p for efficient export mediated by the AAA peroxins [61], the direct mechanistic influence of this modification remains to be investigated. As a consequence of receptor export, ubiquitin has to be removed after or during dislocation by a deubiquitinating enzyme, which represents a new and yet to define step in the PTS-receptor cycle.

Another question still to answer is the identity of the E3-enzymes for poly- and monoubiquitination. The best candidates are Pex2p, Pex10p and Pex12p, as the RING finger motif is the catalytical domain of a subclass of E3-enzymes.

In conclusion, the collected evidence indicates that the energy-dependence of peroxisomal protein import is accounted for by two groups of ATP-dependent enzymatic activities required for the release of the PTS-receptors from the peroxisomal membrane. First, receptor ubiquitination, as ubiquitin

has to be activated by an ATP-dependent step before it is passed onto Pex4p, and second, ATP hydrolysis in the conserved AAA-domains of Pex1p and Pex6p in order to pull the primed Pex5p out of the membrane. Because the mechanism required for PTS-receptor recycling resembles the endoplasmatic reticulum associated degradation (ERAD)-pathway, peroxisomes do not only share the origin of their membranes with the ER, but also the principle of their ubiquitin-based protein targeting system.

Acknowledgements: We are grateful to Sigrid Wüthrich for technical assistance, Wolfgang Schliebs and Wolfgang Girzalsky for the reading of the manuscript and the members of our laboratory for fruitful discussions. This work was supported by the Deutsche Forschungsgemeinschaft (SFB642, Er178/2-4), the FP6 European Union Project 'Peroxisome' (LSHG-CT-2004-512018) and by the Fonds der Chemischen Industrie. We apologize to all the scientists whose work could not be cited due to space limitations.

References

[1] Rhodin, J. (1954) Correlation of ultrastructural organization and function in normal and experimentally changed peroxisomal convoluted tubule cells of the mouse kidney. Dissertation, Aktiebolaget Godvil, Stockholm.
[2] DeDuve, C. (1992) Exploring cells with a centrifuge in: Nobel Lectures in Physiology 1971–1980 (Lindsten, J., Ed.), pp. 152–172, World Scientific Publishing Co., London.
[3] DeDuve, C. and Baudhuin, P. (1966) Peroxisomes (microbodies and related particles). Physiol. Rev. 46, 323–357.
[4] Opperdoes, F.R. (1988) Glycosomes may provide clues to the import of peroxisomal proteins. Trends Biochem. Sci. 13, 255–260.
[5] Poirier, Y., Antonenkov, V.D., Glumoff, T. and Hiltunen, J.K. (2006) Peroxisomal β-oxidation – a metabolic pathway with multiple functions. Biochim. Biophys. Acta 1763, 1413–1426.
[6] Baker, A., Graham, I.A., Holdsworth, M., Smith, S.M. and Theodoulou, F.L. (2005) Chewing the fat: beta-oxidation in signalling and development. Trends Plant Sci. 11, 124–132.
[7] Kersten, S., Desvergne, B. and Wahli, W. (2000) Roles of PPARs in health and disease. Nature 405, 421–424.
[8] Jansen, G.A. and Wanders, R.J. (2006) Alpha-Oxidation. Biochim. Biophys. Acta 1763, 1403–1412.
[9] Reumann, S. and Weber, A.P. (2006) Plant peroxisomes respire the light: some gaps of the photorespiratory C2 cycle have become filled – others remain. Biochim. Biophys. Acta 1763, 1450–1496.
[10] Müller, W.H., van der Krift, T.P., Krouwer, A.J.J., Wosten, H.A.B. and van der Voort, L.H.M. (1991) Localisation of the pathway of the penicillin biosynthesis in *Penicillium chrysogenum*. EMBO J. 10, 489–496.
[11] Wanders, R.J. (2004) Metabolic and molecular basis of peroxisomal disorders: a review. Am. J. Med. Genet. 126A, 355–375.
[12] Terlecky, S.R., Koepke, J.I. and Walton, P.A. (2006) Peroxisomes and aging. Biochim. Biophys. Acta 1763, 1749–1754.
[13] Breidenbach, R.W. and Beevers, H. (1967) Association of the glyoxylate cycle enzymes in a novel subcellular particle from castor bean endosperm. Biochem. Biophys. Res. Commun. 27, 462–469.
[14] Woronin, M. (1864) Zur Entwicklungsgeschichte der *Ascobolus pulcherrimus* Cr. und einiger Pezizien. Abh. Senkenb. Naturforsch. 5, 333–344.
[15] Jedd, G. and Chua, N.H. (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat. Cell Biol. 2, 226–231.
[16] Opperdoes, F.R. and Borst, P. (1977) Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma brucei*: the glycosome. FEBS Lett. 80, 360–364.
[17] Idnurm, A., Giles, S.S., Perfect, J.R. and Heitman, J. (2007) Peroxisome function regulates growth on glucose in the basidiomycete fungus *Cryptococcus neoformans*. Eukaryot. Cell 6, 60–72.
[18] Veenhuis, M., Mateblowski, M., Kunau, W.-H. and Harder, W. (1987) Proliferation of microbodies in *Saccharomyces cerevisiae*. Yeast 3, 77–84.
[19] Erdmann, R., Veenhuis, M., Mertens, D. and Kunau, W.-H. (1989) Isolation of peroxisome-deficient mutants of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 86, 5419–5423.
[20] Erdmann, R., Wiebel, F.F., Flessau, A., Rytka, J., Beyer, A., Fröhlich, K.U. and Kunau, W.-H. (1991) *PAS1*, a yeast gene required for peroxisome biogenesis, encodes a member of a novel family of putative ATPases. Cell 64, 499–510.
[21] Distel, B. et al. (1996) A unified nomenclature for peroxisome biogenesis factors. J. Cell Biol. 135, 1–3.
[22] Vizeacoumar, F.J., Torres-Guzman, J.C., Bouard, D., Aitchison, J.D. and Rachubinski, R.A. (2004) Pex30p, Pex31p and Pex32p form a family of peroxisomal integral membrane proteins regulating peroxisome size and number in *Saccharomyces cerevisiae*. Mol. Biol. Cell 15, 665–677.
[23] van der Zand, A., Braakman, I., Geuze, H.J. and Tabak, H.F. (2006) The return of the peroxisome. J. Cell Sci. 119, 989–994.
[24] Novikoff, A.B. and Shin, W.-Y. (1964) The endoplasmatic reticulum in the golgi zone and ist relations to microbodies, golgiapparatus and autophagic vacuoles in rat liver cells. J. Mikros. Oxford 3, 187–206.
[25] Lazarow, P.B. and Fujiki, Y. (1985) Biogenesis of peroxisomes. Ann. Rev. Cell Biol. 1, 489–530.
[26] Hoepfner, D., Schildknegt, D., Braakman, I., Philippsen, P. and Tabak, H.F. (2005) Contribution of the endoplasmic reticulum to peroxisome formation. Cell 122, 89–95.
[27] Kim, P.K., Mullen, R.T., Schumann, U. and Lippincott-Schwartz, J. (2006) The origin and maintenance of mammalian peroxisomes involves a de novo PEX16-dependent pathway from the ER. J. Cell Biol. 173, 521–532.
[28] Titorenko, V.I. and Rachubinski, R.A. (2000) Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p. J. Cell Biol. 150, 881–886.
[29] Lay, D., Gorgas, K. and Just, W.W. (2006) Peroxisome biogenesis: where Arf and coatomer might be involved. Biochim. Biophys. Acta 1763, 1678–1687.
[30] Mullen, R.T. and Trelease, R.N. (2006) The ER-peroxisome connection in plants: development of the "ER semi-autonomous peroxisome maturation and replication" model for plant peroxisome biogenesis. Biochim. Biophys. Acta 1763, 1655–1668.
[31] Schrader, M. and Fahimi, H.D. (2006) Growth and division of peroxisomes. Int. Rev. Cytol. 255, 237–290.
[32] Marelli, M. et al. (2004) Quantitative mass spectrometry reveals a role for the GTPase Rho1p in actin organization on the peroxisome membrane. J. Cell Biol. 167, 1099–1112.
[33] Vizeacoumar, F.J., Torres-Guzman, J.C., Tam, Y.Y., Aitchison, J.D. and Rachubinski, R.A. (2003) YHR150w and YDR479c encode peroxisomal integral membrane proteins involved in the regulation of peroxisome number, size, and distribution in *Saccharomyces cerevisiae*. J. Cell Biol. 161, 321–332.
[34] Fagarasanu, M., Fagarasanu, A. and Rachubinsk, R.A. (2006) Sharing the wealth: peroxisome inheritance in budding yeast. Biochim. Biophys. Acta 1763, 1669–1677.
[35] Fujiki, Y., Matsuzono, Y., Matsuzaki, T. and Fransen, M. (2006) Import of peroxisomal membrane proteins: the interplay of Pex3p- and Pex19p-mediated interactions. Biochim. Biophys. Acta 1763, 1639–1646.
[36] Vizeacoumar, F.J., Vreden, W.N., Aitchison, J.D. and Rachubinski, R.A. (2006) Pex19p binds Pex30p and Pex32p at regions required for their peroxisomal localization but separate from their peroxisomal targeting signals. J. Biol. Chem. 281, 14805–14812.
[37] Rottensteiner, H., Kramer, A., Lorenzen, S., Stein, K., Landgraf, C., Volkmer-Engert, R. and Erdmann, R. (2004) Peroxisomal membrane proteins contain common Pex19p-binding sites that are an integral part of their targeting signals (mPTS). Mol. Biol. Cell 7, 3406–3417.
[38] Girzalsky, W., Hoffmann, L.S., Schemenewitz, A., Nolte, A., Kunau, W.H. and Erdmann, R. (2006) Pex19p-dependent targeting of Pex17p, a peripheral component of the peroxisomal protein import machinery. J. Biol. Chem. 281, 19417–19425.
[39] Schliebs, W. and Kunau, W.H. (2004) Peroxisome membrane biogenesis: the stage is set. Curr. Biol. 14, R397–R399.

[40] Lambkin, G.R. and Rachubinski, R.A. (2001) *Yarrowia lipolytica* cells mutant for the peroxisomal peroxin Pex19p contain structures resembling wild-type peroxisomes. Mol. Biol. Cell 12, 3353–3364.
[41] Otzen, M., Krikken, A.M., Ozimek, P.Z., Kubatova, E., Nagotu, S., Veenhuis, M. and Van Der Klei, I. (2006) In the yeast *Hansenula polymorpha*, peroxisome formation from the ER is independent of Pex19p, but involves the function of p24 proteins. FEMS Yeast Res. 6, 1157–1166.
[42] Wickner, W. and Schekman, R. (2005) Protein translocation across biological membranes. Science 310, 1452–1456.
[43] Gould, S.J., Keller, G.A., Hosken, N., Wilkinson, J. and Subramani, S. (1989) A conserved tripeptide sorts proteins to peroxisomes. J. Cell Biol. 108, 1657–1664.
[44] Brocard, C. and Hartig, A. (2006) Peroxisome targeting signal 1: is it really a simple tripeptide? Biochim. Biophys. Acta 1763, 1565–1573.
[45] Stanley, W.A., Filipp, F.V., Kursula, P., Schuller, N., Erdmann, R., Schliebs, W., Sattler, M. and Wilmanns, M. (2006) Recognition of a functional peroxisome type 1 target by the dynamic import receptor Pex5p. Mol. Cell 24, 653–663.
[46] Swinkels, B.W., Gould, S.J., Bodnar, A.G., Rachubinski, R.A. and Subramani, S. (1991) A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. EMBO J. 10, 3255–3262.
[47] Marzioch, M., Erdmann, R., Veenhuis, M. and Kunau, W.-H. (1994) *PAS7* encodes a novel yeast member of the WD-40 protein family essential for import of 3-oxoacyl-CoA thiolase, a PTS2-containing protein, into peroxisomes. EMBO J. 13, 4908–4918.
[48] Schliebs, W. and Kunau, W.-H. (2006) PTS2 co-receptors: diverse proteins with common features. Biochim. Biophys. Acta 1763, 1605–1612.
[49] Eckert, J.H. and Erdmann, R. (2003) Peroxisome biogenesis. Rev. Physiol. Biochem. Pharmacol. 147, 75–121.
[50] Schäfer, A., Kerssen, D., Veenhuis, M., Kunau, W.H. and Schliebs, W. (2004) Functional similarity between the peroxisomal PTS2 receptor binding protein Pex18p and the N-terminal half of the PTS1 receptor Pex5p. Mol. Cell Biol. 24, 8895–8906.
[51] Kiel, J.A., Veenhuis, M. and Van Der Klei, I. (2006) *PEX* genes in fungal genomes: common, rare or redundant. Traffic 7, 1291–1303.
[52] Williams, C., van den Berg, M. and Distel, B. (2005) *Saccharomyces cerevisiae* Pex14p contains two independent Pex5p binding sites, which are both essential for PTS1 protein import. FEBS Lett. 579, 3416–3420.
[53] Kerssen, D., Hambruch, E., Klaas, W., Platta, H.W., de Kruijff, B., Erdmann, R., Kunau, W.-H. and Schliebs, W. (2006) Membrane association of the cycling peroxisome import receptor Pex5p. J. Biol. Chem. 281, 27003–27015.
[54] Platta, H.W., Grunau, S., Rosenkranz, K., Girzalsky, W. and Erdmann, R. (2005) Functional role of the AAA peroxins in dislocation of the cycling PTS1 receptor back to the cytosol. Nat. Cell Biol. 7, 817–822.
[55] Urquhart, A.J., Kennedy, D., Gould, S.J. and Crane, D.I. (2000) Interaction of Pex5p, the Type 1 peroxisome targeting signal receptor, with the peroxisomal membrane proteins Pex14p and Pex13p. J. Biol. Chem. 275, 4127–4136.
[56] Gouveia, A.M., Reguenga, C., Oliveira, M.E., Sa-Miranda, C. and Azevedo, J.E. (2000) Characterization of peroxisomal Pex5p from rat liver: Pex5p in the Pex5p-Pex14p membrane complex is a transmembrane protein. J. Biol. Chem. 275, 32444–32451.
[57] Dammai, V. and Subramani, S. (2001) The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisomal matrix and recycled to the cytosol. Cell 105, 187–196.
[58] Kunau, W. (2001) Peroxisomes: the extended shuttle to the peroxisome matrix. Curr. Biol. 11, R659–R662.
[59] Nair, D.M., Purdue, P.E. and Lazarow, P.B. (2004) Pex7p translocates in and out of peroxisomes in *Saccharomyces cerevisiae*. J. Cell Biol. 167, 599–604.
[60] Leon, S., Zhang, L., McDonald, W.H., Yates, J.r., Cregg, J.M. and Subramani, S. (2006) Dynamics of the peroxisomal import cycle of PpPex20p: ubiquitin-dependent localization and regulation. J. Cell Biol. 172, 67–78.
[61] Erdmann, R. and Schliebs, W. (2005) Peroxisomal matrix protein import: the transient pore model. Nat. Rev. Mol. Cell Biol. 6, 738–742.
[62] Rayapuram, N. and Subramani, S. (2006) The importomer – a peroxisomal membrane complex involved in protein translocation into the peroxisome matrix. Biochim. Biophys. Acta 1763, 1613–1619.
[63] Agne, B. et al. (2003) Pex8p. An intraperoxisomal organizer of the peroxisomal import machinery. Mol. Cell 11, 635–646.
[64] Chang, C.C., Warren, D.S., Sacksteder, K.A. and Gould, S.J. (1999) PEX12 Interacts with PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import. J. Cell Biol. 147, 761–774.
[65] Dodt, G. and Gould, S.J. (1996) Multiple *PEX* genes are required for proper subcellular distribution and stability of Pex5p, the PTS1 receptor: evidence that PTS1 protein import is mediated by a cycling receptor. J. Cell Biol. 135, 1763–1774.
[66] Miyata, N. and Fujiki, Y. (2005) Shuttling mechanism of peroxisome targeting signal Type 1 receptor Pex5: ATP-independent import and ATP-dependent export. Mol. Cell Biol. 25, 10822–10832.
[67] Otera, H. et al. (2000) The mammalian peroxin Pex5pL, the longer isoform of the mobile peroxisome targeting signal (PTS) Type 1 transporter, translocates the Pex7p-PTS2 protein complex into peroxisomes via its initial docking site, Pex14p. J. Biol. Chem. 275, 21703–21714.
[68] Kurochkin, I.V., Mizuno, Y., Konagaya, A., Sakaki, Y., Schonbach, C. and Okazaki, Y. (2007) Novel peroxisomal protease Tysnd1 processes PTS1- and PTS2-containing enzymes involved in beta-oxidation of fatty acids. EMBO J. 26, 835–845.
[69] Imanaka, T., Small, G.M. and Lazarow, P.B. (1987) Translocation of acyl-CoA oxidase into peroxisomes requires ATP hydrolysis but not a membrane potential. J. Cell Biol. 105, 2915–2922.
[70] Oliveira, M.E., Gouveia, A.M., Pinto, R.A., Sa-Miranda, C. and Azevedo, J.E. (2003) The energetics of Pex5p-mediated peroxisomal protein import. J. Biol. Chem. 278, 39483–39488.
[71] Rosenkranz, K., Birschmann, I., Grunau, S., Girzalsky, W., Kunau, W.-H. and Erdmann, R. (2006) Functional association of th AAA-complex and the peroxisomal importomer. FEBS J. 273, 3015–3804.
[72] Jentsch, S. and Rumpf, S. (2007) Cdc48 (p97): a 'molecular gearbox' in the ubiquitin pathway? Trends Biochem. Sci. 32, 6–11.
[73] Gabaldon, T., Snel, B., van Zimmeren, F., Hemrika, W., Tabak, H.F. and Huynen, M.A. (2006) Origin and evolution of the peroxisomal proteome. Biol. Direct 1, 1–14.
[74] Shiozawa, K. et al. (2004) Structure of the N-terminal domain of PEX1 AAA-ATPase: characterization of a putative adaptor-binding domain. J. Biol. Chem. 279, 50060–50068.
[75] Park, S., Isaacson, R., Kim, H.T., Silver, P.A. and Wagner, G. (2005) Ufd1 exhibits the AAA-ATPase fold with two distinct ubiquitin interaction sites. Structure 13, 995–1005.
[76] Platta, H.W., Girzalsky, W. and Erdmann, R. (2004) Ubiquitination of the peroxisomal import receptor Pex5p. Biochem. J. 384, 37–45.
[77] Purdue, P.E. and Lazarow, P.B. (2001) Pex18p is constitutively degraded during peroxisome biogenesis. J. Biol. Chem. 276, 47684–47689.
[78] Kragt, A., Voorn-Brouwer, T.M., Van den Berg, M. and Distel, B. (2005) The *Saccharomyces cerevisiae* peroxisomal import receptor Pex5p is monoubiquitinated in wild type cells. J. Biol. Chem. 280, 7867–7874.
[79] Kiel, J.A., Emmrich, K., Meyer, H.E. and Kunau, W.H. (2005) Ubiquitination of the PTS1 receptor, Pex5p, suggests the presence of a quality control mechanism during peroxisomal matrix protein import. J. Biol. Chem. 280, 1921–1930.
[80] Kiel, J.A., Otzen, M., Veenhuis, M. and van der Klei, I.J. (2005) Obstruction of polyubiquitination affects PTS1 peroxisomal matrix protein import. Biochim. Biophys. Acta 1745, 176–186.
[81] Zolman, B.K. and Bartel, B. (2004) An Arabidopsis indole-3-butyric acid-response mutant defective in PEROXIN6, an apparent ATPase implicated in peroxisomal function. Proc. Natl. Acad. Sci. USA 101, 1786–1791.
[82] van der Klei, I.J., Hilbrands, R.E., Kiel, J.A.K.W., Rasmussen, S.W., Cregg, J.M. and Veenhuis, M. (1998) The ubiquitin-conjugating enzyme Pex4p of *Hansenula polymorpha* is required for efficient functioning of the PTS1 import machinery. EMBO J. 17, 3608–3618.

[83] Kiel, J.A. et al. (1999) *Hansenula polymorpha* Pex1p and Pex6p are peroxisome-associated AAA proteins that functionally and physically interact. Yeast 15, 1059–1078.
[84] Collins, C.S., Kalish, J.E., Morrell, J.C., McCaffery, J.M. and Gould, S.J. (2000) The peroxisome biogenesis factors Pex4p, Pex22p, Pex1p, and Pex6p act in the terminal steps of peroxisomal matrix protein import. Mol. Cell Biol. 20, 7516–7526.
[85] Platta, H.W., El Magraoui, F., Schlee, D., Grunau, S., Girzalsky, W. and Erdmann, R. (2007) Ubiquitination of the peroxisomal import receptor Pex5p is required for its recycling. J. Cell. Biol. 177, in press.
[86] Mukhopadhyay, D. and Riezman, H. (2007) Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 315, 201–205.
[87] Wiebel, F.F. and Kunau, W.-H. (1992) The PAS2 protein essential for peroxisome biogenesis is related to ubiquitin-conjugating enzymes. Nature 359, 73–76.
[88] Koller, A., Snyder, W.B., Faber, K.N., Wenzel, T.J., Rangell, L., Keller, G.A. and Subramani, S. (1999) Pex22p of *Pichia pastoris*, essential for peroxisomal matrix protein import, anchors the ubiquitin-conjugating enzyme, Pex4p, on the peroxisomal membrane. J. Cell Biol. 146, 99–112.
[89] Leon, S. and Subramani, S. (2007) A conserved cysteine residue of *Pichia pastoris* is essential for its recycling from the peroxisome to the cytosol. J. Biol. Chem. 282, 7424–7430.